(d) purifying the resultant hybrid cell by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection.

Please add the following new claims:

29. (New) The method of claim 14, wherein said accessory factor is B7.

30. (New) The method of claim 18, wherein said accessory factor is B7.

31. (New) The method of claim 19, wherein said accessory factor is B7.

## REMARKS

### Status of the Claims

By this amendment, claim 16 is canceled, claims 14, 15, 18 and 19 are amended and claims 29-31 are added. Accordingly, upon entry of this Amendment, claims 1-15 and 17-31 will be pending in the application, with claims 1-13 and 23-28 withdrawn from consideration as a result of a Restriction Requirement, and claims 14, 15, 17-22 and 29-31 presented for examination on the merits.

Support for the amendments to claim 14, 18 and 19 is found in originally filed claim 6 and in the specification on page 6, lines 8-17. Support for the amendment to claim 15 is found on page 8, lines 6-13 of the specification. Support for the amendment to claim 18 is found on page 8, lines 3-5 of the specification. Support for newly added claims 29-31 is found on page 12, lines 1-16.

Because the foregoing amendments do not introduce new matter, entry thereof by the examiner is respectfully requested.

### Claim Rejections - 35 U.S.C. § 112, First Paragraph

Claims 14-22 are rejected by the Examiner under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement and written description. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that the method of claims 14, 18 and 19 embrace any condition for inducing cell fusion and any method of purifying hybrid cells. However, the Examiner

asserts that the specification allegedly fails to teach conditions other than polyethylene glycols that could induce cell fusion and purification methods other than FACS. The Examiner further asserts that a person of ordinary skill in the art would not know how to practice the invention commensurate in scope with the claims, other than the method recited in the specification.

With respect to conditions for inducing cell fusion, Applicants direct the Examiner's attention to page 9, lines 24-25 where the following statement is made: "Such fusion-promoting conditions are well known to the artisan, and typically involve the addition of an agent that promotes cells fusion.

While the invention contemplates any agent that meets these characteristics, exemplary useful agents are polymeric compounds, like polyethylene glycols." A person of ordinary skill in the art would have been aware of various reaction conditions for inducing cell fusion other than those employing polyethylene glycols and would not have had to undertake undue experimentation in order to determine suitable conditions for inducing cell fusion. Attached as exhibits 1 and 2 are Chiu, D. T. A microfluidics platform for cell fusion. *Curr. Opin. Chem. Biol.* 2001, 5(5):609-612; and Kerkis AYu, Zhdanova, N. S. "Formation and ultrastructure of somatic cell hybrids". *Electron Microsc. Rev.* 1992, 5(1): 1-24, respectively. These references describe techniques for cell fusion with which a person of ordinary skill in the art would have been familiar. For example, Chiu et al. describes electrofusion and also states in the left panel on page 609 "There are a number of methods for carrying out cell-cell fusion *in vitro*, including the use of chemical such as polyethylene glycol (PEG) [4, 5], the use of focused laser beams (laser-induced fusion) [6, 7], and the application of pulsed electric fields (electrofusion) [8, 9]." Additionally, Kerkis et al. describes the use of Sendai virus as a fusogenic agent to fuse cells.

With respect to purification methods, while Applicants respectfully disagree with the Examiner's assertion, Applicants have amended the claims in order to expedite prosecution. Applicants have amended claims 14, 18 and 19 to recite that the purification is accomplished by cell sorting, wherein the cell sorting does not involve antibiotic or metabolic selection.

The present specification provides support for purifying the hybrid cells by cell sorting without the need for antibiotic or metabolic selection. *See,* for example, page 6, lines 8-17 of the present specification.

Additionally, those of ordinary skill in the art would not have had to undertake undue experimentation in order to determine suitable cell sorting methods for purifying hybrid cells. For example, fluorescence activated cell sorting is provided as an exemplary cell sorting method. Thus, the present specification provides sufficient written description and enablement for purifying the hybrid cell by cell sorting, wherein the cell sorting does not involve antibiotic or metabolic selection.

**Claim Rejections - 35 U.S.C. § 112, Second Paragraph**

Claims 14-18 are rejected by the Examiner under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that the method recited in claim 14 is incomplete because it allegedly fails to recite a positive method step. Applicants have amended claim 14 to recite that the purification is accomplished by cell sorting, wherein the cell sorting does not involve antibiotic or metabolic selection. Therefore, claim 14, as amended, satisfies the requirements of 35 U.S.C. § 112, second paragraph.

The Examiner asserts that in claim 15, there is insufficient antecedent basis for "said purification". Applicants have amended claim 15 to recite "said purifying", for which there is sufficient antecedent basis.

The Examiner asserts that in claim 15, it is unclear when the labeling of each of the fusion partner cells with a different fluorescent dye occurs. Applicants direct the Examiner's attention to page 8, lines 6-13 where this pre-fusion labeling step is discussed. Applicants have amended claim 15 to clarify that the labeling occurs prior to bringing said cells into contact under conditions that promote cell fusion.

The Examiner asserts that in claim 18, the phrase "purifying the resultant hybrid cell in less than 48 hours" is unclear because the phrase could be interpreted as the purification process takes less than 48 hours or the cells are purified within 48 hours from the starting point of fusion. Applicants direct the Examiner's attention to page 8, lines 3-5 where it states "In general, the purification is accomplished in a relatively short period of time, for example, in less than about 24 to 48 hours, after exposure to conditions that promote fusion. Applicants have amended claim 18 to recite "purifying the resultant hybrid cell in less than 48 hours after exposure to conditions that promote fusion."

The Examiner asserts that in claim 18, it appears that the term "under" is missing after "contact" in line 2. Applicants have amended claim 18 to recite "under" after the word "contact" .

The Examiner asserts that in claims 14, 18 and 19, the phrase "under conditions that promote cell fusion" is unclear because the claims do not indicate the conditions that promote cell fusion. Applicants respectfully disagree with the Examiner. As discussed above, the phrase "under conditions that promote cell fusion" would have been clear to a person of ordinary skill in the art because such a person would have been familiar with various methods for achieving this goal. Claim 14 recites two positive method steps — bringing two cells into contact and purifying. Therefore, claim 14 satisfies the requirements of 35 U.S.C. § 112, second paragraph.

The Examiner asserts that in claims 16 and 21, the phrase "an antigen presenting cell that lacks an accessory factor required to generate a positive immune response" is unclear because the claims and specification allegedly fail to indicate the accessory factor. Applicants note that accessory factors are described throughout the specification. *See, for example*, page 4, lines 17-23; page 5, lines 4-12; page 12, lines 1-16; and page 14, lines 3-8. The specification mentions B7 as an exemplary accessory factor and other accessory factors are well known to those of skill in the art.

**Claim Rejections - 35 U.S.C. § 102**

A. Claims 14-19 are rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Koolwijk et al. (*Hybridoma* 1988; 7:217-225). The Examiner asserts that the method of Koolwijk et al. is the same as the present method. The Examiner assumes that the purification of Koolwijk et al. took less than 48 hours, although Koolwijk et al. fails to specify a time frame. The Examiner bases his assumption on the fact that Koolwijk et al. allegedly uses the same fusion and selection technique taught in the present specification. Applicants respectfully request reconsideration and withdrawal of the rejection.

Present Invention

The present claims, as amended, are directed toward a method of preparing a hybrid cell, comprising fusing two different cells (wherein one of said cells is selected from the group consisting of a macrophage, a dendritic cell and an antigen presenting cell that lacks an accessory factor required to generate a positive immune response) and then purifying the resultant hybrid cell by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection.

A feature of the present invention is that the heterogeneity/diversity of the starting cell population is preserved in the hybrid cells. The relevance of this feature can be explained by way of example, such as an embodiment of the present invention wherein the resulting population of hybrid cell is used as a vaccine for treating tumors. However, this feature applies to all embodiments of the present invention. In the embodiment wherein the resultant population of hybrid cells is a vaccine for treating tumors, one of the cells in the hybrid cell (the "target" cell) is a tumor cell and the second cell is a different type of cell, such as an antigen presenting cell. It is well known in the art that a tumor is often comprised of heterogeneous sub-populations of cells. Maintaining this heterogeneity in the population of hybrid cells that results from the fusion of the tumor cells with the second cells is important because if a vaccine is administered wherein only a fraction of the sub-populations of tumor cells are represented, an immune response will only be triggered against the represented types of tumor cells. An outcome of using such a vaccine on a patient is that the sub-populations of

tumor cells not represented in the vaccine grow stronger and multiply faster. To prevent such an outcome, it is important to maintain the heterogeneity/diversity of tumor cells used as starting material in the population of hybrid cells used as a vaccine. Classical selection methods, such as those involving the use of antibiotic or metabolic selection, fail to preserve the heterogeneity/diversity of starting material cells because some of the sub-populations of starting material cells are eliminated by the selection step. Therefore, purification methods involving the use of antibiotic or metabolic selection are not suitable for the present invention.

Not only is it important to maintain the heterogeneity/diversity of the starting cell population, it is also important to administer a purified preparation of fused cells to a patient. Attached herewith as Exhibit 3 is Li et al., *Cancer Immunol. Immunother.* **50** 456-462 (2001). The authors compared results from cytotoxicity assay, interferon-γ production and *in vivo* lung tumor metastasis studies and showed that purified hybrid cells are more effective than fusion mixtures in stimulating antitumor activity. *See* Abstract. Thus, the cell sorting method employed in the method of the present invention is a feature that improves the efficacy of the vaccine because it preserves the heterogeneity/diversity of the starting material cells and eliminates unfused starting material cells from the resultant hybrid cells.

Koolwijk et al.

Koolwijk et al. is directed to a method of preparing hybrid hybridomas that produce bi-specific monoclonal antibodies, comprising contacting a first hybridoma cell with a green fluorescent, contacting a second hybridoma cell with a red fluorescent and fusing the cells with polyethylene glycol 4000.

Distinctions Over Koolwijk et al.

The present invention is not anticipated by Koolwijk et al. because Koolwijk et al. fails to teach each and every limitation of the present invention. While Koolwijk et al. discloses the fusion of two hybridoma cells, the present invention, in contrast, is directed to fusing a macrophage, a dendritic cell or an antigen presenting cell that lacks an accessory

factor required to generate a positive immune response with at least one other cell. Since Koolwijk et al. fails to disclose a fusion between a macrophage, a dendritic cell or an antigen presenting cell that lacks an accessory factor required to generate a positive immune response and at least one other cell, the present claims are not anticipated by Koolwijk et al.

B. Claim 14 is rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Wagner et al. (*Cytogenet. Cell. Genet.*, 1997; 76:172-175). Applicants respectfully request reconsideration and withdrawal of the rejection.

Wagner et al.

Wagner et al. disclose a somatic cell hybrid panel consisting of seven hybrids with translocation breakpoints spanning the region of 17q23→q25. Selection of hybrid clones was achieved using hypoxanthine-aminopterin-thymidine (HAT) medium to retain human chromosome 17 or the derivative chromosomes bearing the TK1 locus on 17q25. See left panel of page 173.

Distinctions Over Wagner et al.

The present invention is not anticipated by Wagner et al. because Wagner et al. utilizes a selection step that is outside the scope of the present claims and is not suitable for the present invention. While the present claims state that the resultant hybrid cell is purified by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection, Wagner et al., on the other hand, select their hybrid clones in hypoxanthine-aminopterin-thymidine (HAT) medium. When grown in HAT medium, only cell lines expressing both hypoxanthine phosphoribosyl transferase (HPRT+) and thymidine kinase (TK+) can survive. Therefore, not only is the method of Wagner et al. different from the method of the present invention, it teaches away from the method of the present invention. As discussed above, a feature of the present invention is preserving the heterogeneity/diversity of the starting material cells and purifying the hybrid cells from the fusion mixture. If the selection method utilized by Wagner et al. were used as the purification method of the present invention, the

diversity of the starting cell sub-populations would be lost. Therefore, the method of Wagner et al., which involves classical selection methods, does not anticipate the present invention.

C. Claim 18 is rejected by the Examiner under 35 U.S.C. § 102 as being anticipated by Lespagnard et al. (*Int. J. Cancer*, 1998; 76:250-258). Applicants respectfully request reconsideration and withdrawal of the rejection.

Lespagnard et al.

Lespagnard et al. teach a method comprising fusing dendritic cells with mastocytoma cells and purifying the cells in less than 48 hours (abstract and flow cytometry section on page 252). Selection of fused cells was achieved by suspended the fusion mixture in selection medium (RPMI-1640 containing HAT, 10% FCS and additives) and plating the cells.

Distinctions Over Lespagnard et al.

As discussed above with respect to Wagner et al., the present invention is not anticipated by Lespagnard et al. because Lespagnard et al. utilizes a selection step that is outside the scope of the present claims and is not suitable for the present invention. While the present claims state that the resultant hybrid cell is purified by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection, Lespagnard et al., in contrast, select for hybrid cells by growing the fusion mixture in a medium containing hypoxanthine-aminopterin-thymidine (HAT). Only cell lines expressing both hypoxanthine phosphoribosyl transferase (HPRT+) and thymidine kinase (TK+) can survive. Therefore, not only is the method of Lespagnard et al. different from the method of the present invention, it teaches away from the method of the present invention. As discussed above, a feature of the present invention is preserving the heterogeneity/diversity of the starting material cells and purifying the hybrid cells from the fusion mixture. If the selection method utilized by Lespagnard et al. were used as the purification method of the present invention, the diversity of the starting cell sub-populations would be lost. Therefore, the method Lespagnard et al., which involves classical selection methods, does not anticipate the present invention.

**Claim Rejections - 35 U.S.C. § 103**

A. Claims 14-22 are rejected by the Examiner under 35 U.S.C. § 103 as being unpatentable over Koolwijk et al. as applied to claim 14-19 above, and further in view of Horan et al. (U.S. Patent No. 4,859,584) and Deka et al. (U.S. Patent No. 6,197,593). Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that although Koolwijk et al do not teach cyanine dyes as dyes that can be used to label fusion partner cells to select and purify the fused cells, it would have been obvious for a person of ordinary skill in the art to use cyanine dyes because Horan et al. teach that some cyanine dyes are more suitable for *in vitro* and particularly *in vivo* use (columns 1 & 2) and Deka et al. teach that cyanine dyes (SYTO and TOTO serials) can be used for distinguishing between different cell populations (abstract and figures). The Examiner asserts that it would have been obvious for a person of ordinary skill in the art to substitute the red and green fluorescent dyes with now available cyanine dyes with reasonable expectation of success.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references (or references when combined) must teach or suggest all the claim limitations. *See* MPEP 2142.

In the present case, the combined teachings of Koolwijk et al., Horan et al. and Deka et al. do not teach or suggest all of the claim limitations. As discussed above, Koolwijk et al. discloses the fusion of two hybridoma cells. Koolwijk et al. fails to teach or disclose fusing a macrophage, a dendritic cell or an antigen presenting cell that lacks an accessory factor required to generate a positive immune response with at least one other cell. It would not have been obvious to a person of ordinary skill in the art, familiar with the teachings of Koolwijk et al., to substitute a macrophage, a dendritic cell or an antigen presenting cell that lacks an accessory factor required to generate a positive immune response for one of the

hybridoma cells because Koolwijk et al. is directed to producing bi-specific monoclonal antibodies. No where in Koolwijk et al. is it taught or suggested to use any cell other than hybridoms as starting material cells. The teachings of Horan et al. and Deka et al. do not cure the deficiencies of Koolwijk et al. Neither Horan et al. nor Deka et al. teach or suggest fusing a macrophage, a dendritic cell or an antigen presenting cell that lacks an accessory factor required to generate a positive immune response with at least one other cell. Therefore, the present invention is not obvious over Koolwijk et al., Horan et al. and Deka et al.

B. Claims 14-22 are rejected under 35 U.S.C. § 103 as being unpatentable over Koolwijk et al., Horan et al. and Deka et al. as applied to claim 14-22 above, and further in view of Gong et al. (*Nature* 1997; 3:558-561). Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that although Koolwijk et al, Horan et al., and Deka et al. do not teach fusing tumor cells and dendritic cells, the fact that Gong et al. teaches fusion between dendritic cells and carcinoma cells for antitumor activity and because Gong et al. use a fluorescent labeled antibody for cell selection, it would have been obvious for a person of ordinary skill in the art to combine the teachings of Koolwijk et al, Horan et al., Deka et al and Gong et al. to arrive at a process for purification of dendritic-tumor cell hybrids with a reasonable expectation of success.

The present invention is not obvious over the combined teachings of Koolwijk et al, Horan et al., and Deka et al. because these references fail to teach or suggest every limitation of the claimed invention. The present invention is not obvious over the combined teachings of Koolwijk et al., Horan et al., Deka et al., and Gong et al. because Gong et al. teaches away from the present invention. Gong et al. utilized classical selection methods to identify fused cells, as evidence by the fact that the fused cells were plated in the presence of HAT medium. *See* bottom right panel on page 560 of Gong et al. As discussed above, a feature of the present invention is preserving the heterogeneity/diversity of the starting material cells and purifying the hybrid cells from the fusion mixture. If the selection method utilized by Gong et al. were used as the purification method of the present invention, the diversity of the

starting cell sub-populations would be lost. Since Gong et al. uses a purification method that is outside the scope of the present claims and teaches a way from the present invention, the present invention is not obvious over the combined teachings of Koolwijk et al, Horan et al., Deka et al. and Gong et al.

## CONCLUSION

As the above-presented amendments and remarks address and overcome all of the rejections presented by the Examiner, withdrawal of the rejections and allowance of the claims are respectfully requested.

If the Examiner has any questions concerning this application, he or she is requested to contact the undersigned.

Respectfully submitted,

Date October 24, 2002

By _____

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

14. (Amended) A method of preparing a hybrid cell, comprising:

(a) bringing at least two different cells into contact under conditions that promote cell fusion, wherein one of said cells is selected from the group consisting of a macrophage, a dendritic cell and an antigen presenting cell that lacks an accessory factor required to generate a positive immune response, and then

(b) purifying the resultant hybrid [without antibiotic or metabolic selection] by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection.

15. (Amended) A method according to claim 14, further comprising labeling each of said different cells with a different fluorescent dye prior to bringing said cells into contact under conditions that promote cell fusion, wherein said [purification] purifying is accomplished using fluorescence activated cell sorting.

18. (Amended) A method of preparing a hybrid cell, comprising:

(a) bringing two different cells into contact conditions that promote cell fusion, wherein one of said cells is selected from the group consisting of a macrophage, a dendritic cell and an antigen presenting cell that lacks an accessory factor required to generate a positive immune response, and

(b) purifying the resultant hybrid cell by cell sorting in less than 48 hours, after exposure to conditions that promote fusion, wherein said cell sorting does not involve antibiotic or metabolic selection.

19. A method of preparing a hybrid cell, comprising:

(a) contacting a first cell with a first dye,

(b) contacting a second cell with a second dye,

(c) contacting said first and second cells with one another under conditions that promote cell fusion, wherein said first cell or said second cell is selected from the group consisting of a macrophage, a dendritic cell and an antigen presenting cell that lacks an accessory factor required to generate a positive immune response, and

(d) purifying the resultant hybrid cell by cell sorting, wherein said cell sorting does not involve antibiotic or metabolic selection.